the segmenting germinal area, by the parablast of mesoblastic ova.*

I was under the impression when these observations were made, that fat had never been found, in this form, in the placenta before. I find that I am to some extent anticipated by a paper in the 'Archiv für Gynaekologie,' February, 1896.† One of the authors (Aschoff) wished to examine a malignant uterine growth, which he believed to be of the nature of *Deciduoma malignum*. Before doing so, he examined several specimens of young human ova, in order, as he says, to learn something of the structure of growing chorionic villi. Some of the specimens he hardened in Flemming's solution, and in all of these he found fat in the plasmodial layer of the villi. Aschoff's description of the fat deposit agrees very closely with that already given of my own specimen. "An den Flemmingschen Präparaten ist das Syncytium dadurch ausgezeichnet, dass es in seiner Randzone eine dichte Anhäufung feinster Fetttröpfchen trägt. Dieselbe sind bald sehr fein, bald grobkörnig, aber in den betreffenden Abschnitten des Syncytiums stets von gleicher Grösse . . . . . . Die Fetttröpfchen überall sich finden, wo Chorionepithelzellen, in directesten Stoffwechselaustausch mit den Intervillösenräumen treten" (p. 531).

Aschoff scarcely appreciates the physiological importance of the observation, but there can be no doubt that his observations and my own are mutually confirmatory.

"Note on the Larva and the Postlarval Development of *Leucosolenia variabilis*, H. sp., with Remarks on the Development of other Asconidæ." By E. A. MINCHIN, M.A., Fellow of Merton College, Oxford. Communicated by Professor E. RAY LANKESTER, F.R.S. Received April 25,—Read May 21, 1896.

Introductory Remarks.

Through the kind hospitality of Professor de Lacaze-Duthiers, I was able to spend the spring and summer of last year at the marine laboratories of Banyuls-sur-Mer and Roscoff, where I was chiefly engaged in studying the embryology of the Ascons. In Banyuls I obtained the larvæ of *Leucosolenia cerebrum*, H. sp., in June, and of *L. reticulum*, O.S. sp., in July. In Roscoff I found the larvæ of *L. variabilis*, H. sp., all through August and the early part of September,

* "Formation of the Germinal Layers in Teleostei," 'Roy. Soc. Edin. Trans.,' 1896.

† "Ueber bösartige Tumoren der Chorionzotten," Apfelstedt und Aschoff.

and of *L. coriacea*, Mont. sp., in September. Owing to the inexperience with which I approached the difficult task of rearing these larvæ, my results are not so complete in all details as I could wish, but in the case of *L. variabilis* I was able to obtain a more or less perfect developmental series, and in the other three species I was able to make out satisfactorily the main points in the metamorphosis, especially the important question of the relation between the cell-layers of the larva and those of the adult. I hope to bring my investigations to completion during the present year, but, in the meantime, the results obtained seemed to me of sufficient importance to form the subject of a preliminary note. The material which I collected and preserved was further studied at Munich, in the laboratory of Professor Richard Hertwig, to whom I am indebted for much kind help and advice, as well as hospitality.

The Development of Leucosolenia variabilis (Ascandra variabilis, *H.*).

The larvæ of *L. variabilis* are of the so-called amphiblastula type, but in many respects more primitive than the amphiblastula larva hitherto described in other Calcarea. The minute larvæ (70—80 μ in length, 50—60 μ in breadth) leave the mother sponge by the osculum, and at once rise to the surface of the water, where they swim for about twenty-four hours. They then sink to the bottom, where, after swimming about slowly for twelve to twenty-four hours more, they fix themselves and undergo metamorphosis. The larval life thus lasts for thirty-six to forty-eight hours.

The oval larva (figs. 1 and 2)* is divided into an anterior region composed of ciliated cells and a posterior region composed of non-ciliated granular cells. The centre of the transparent larva is occupied by a conspicuous mass of yellowish-brown pigment. The ciliated cells are slender and elongated, reaching from the pigment to the surface of the body. Each cell bears a single flagellum, and the body of the cell is divided into an internal refractile portion and an external granular portion. These two portions of the cell are so distinct in the living object that a superficial examination gives the impression of an internal layer of refractile cells covered by an external granular layer, but by more careful investigation it is easy to make out that these two apparent layers are merely parts of a single layer of cells. The ciliated cells situated more posteriorly entirely lack the refractile inner portion, and appear granular throughout. They are also slightly broader, and have more convex outer surfaces than the other ciliated cells, forming an equatorial zone of *intermediate cells*, not very distinct in the living object. The

* Figs. 1—6 represent the development of *L. variabilis*, × 1000 diameters. All but 1 and 2 are semidiagrammatic and combined from different preparations.

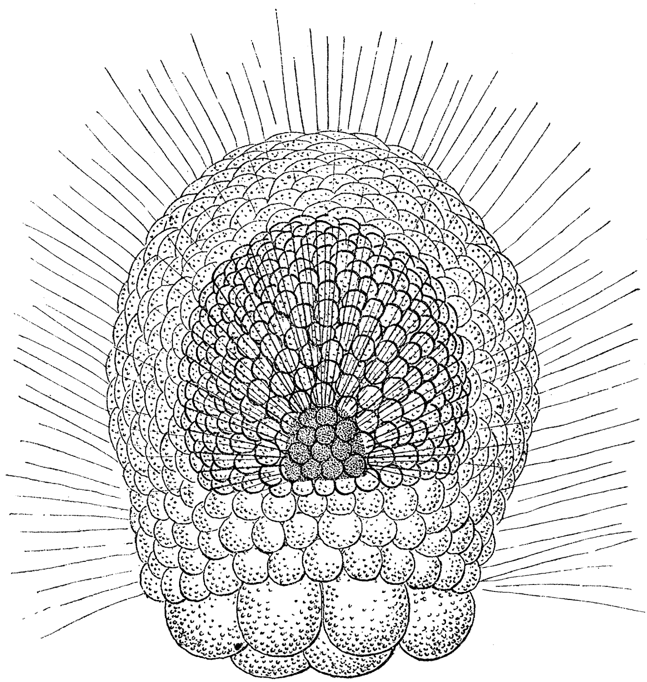

Fig. 1.—Newly hatched larva.

region of the intermediate cells is generally marked by a slight constriction, giving a waist, as it were, to the larvæ. The granular cells are much fewer in number than the other elements, and are also of much larger size, but there are gradations in this respect, those placed at the posterior pole being much larger than those which border upon the intermediate cells.

During the free-swimming larval period, considerable changes take place in the relative proportions of the different parts of the larvæ. In the newly hatched larva (fig. 1) the anterior ciliated region is relatively large, with a very broad granular border to the cells, and the posterior granular cells are few in number. The number of granular cells now increases at the expense of the ciliated cells. Some of the ciliated cells, by absorption of the internal refractile portion of the cell, become intermediate cells, and these, in their turn, absorb their flagellum, increase in size, and become granular cells. This process goes on *pari passu* with a decrease in the granular border of the ciliated cells. In the larva of about twenty-four hours (fig. 2), the granular cells form a mass equal to that of the ciliated cells, and the latter have now a very narrow granular border. In

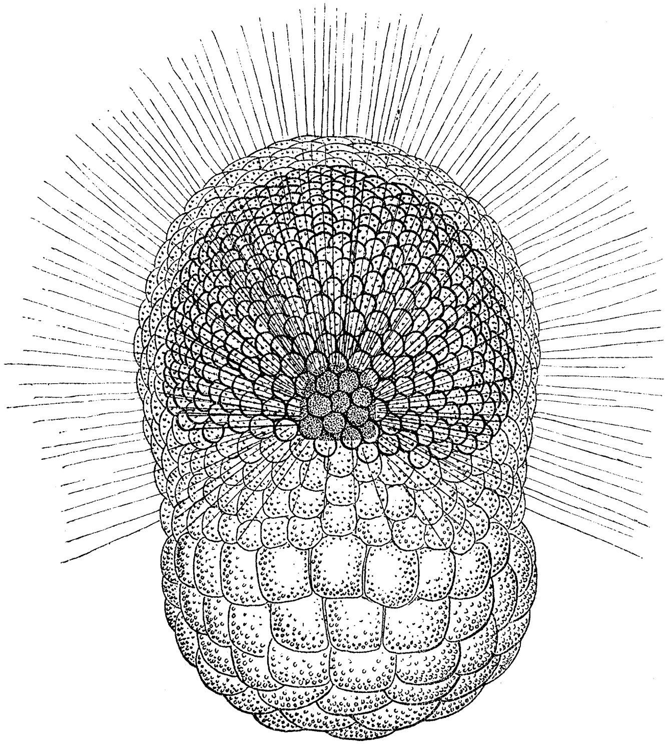

FIG. 2.—Larva of second day.

short, granular cells are formed during larval life by modification of ciliated cells, the intermediate cells being a stage in this process.

Sections of larvæ confirm and amplify the results obtained from a study of the living object (fig. 3). The inner portion of each ciliated cell, which in life appeared refractile, is seen to contain a series of vacuole-like structures, containing granular masses suspended in their interior. At the junction between the internal vacuolated and external granular portions of the cell is situated the opaque and deeply staining nucleus, which has a form like an onion, and is continued externally into the flagellum. Often the inner side of the nucleus is indented by the vacuole beneath it, sometimes to such an extent that the nucleus has the form of a crescent in section. The intermediate cells are very distinct in sections, and by some methods of preservation and staining, *e.g.*, osmic acid followed by picrocarmine, their protoplasm takes up the stain in a remarkable manner, so that larvæ treated in this way appear to have a brightly coloured equatorial zone. They lack the vacuolated inner portion, characteristic of the

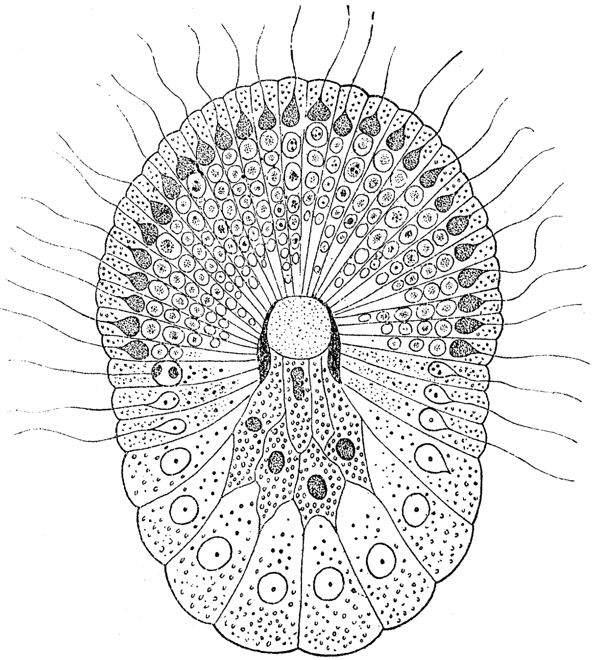

Fig. 3.—Longitudinal section of larva.

ciliated cells proper, and their nuclei are larger and paler with one or two nucleoli. The nucleus of the first intermediate cell freqnently presents a curious appearance, being swollen out into a large vesicular structure containing two or three chromatin masses. This condition is apparently in connexion both with a process of rearrangement of the chromatin and with the absorption of the vacuoles. The granular cells are arranged in a single layer, and have large pale nuclei with nucleoli. Often the nucleus of the cell nearest the intermediate cells has a pointed outer end, evidently indicating the former connexion with the flagellum.

Sections reveal a remarkable set of structures in connexion with the central pigment, which is now seen to have the form of a tube, open in front and behind, and enclosing a rounded, lens-like body, apparently a gelatinous mass filling the central cavity, the remnant, doubtless, of the segmentation cavity. Behind these bodies are a number of cells with coarse granules and small, very opaque, deeply staining nuclei.* One of these cells is placed in the longitudinal axis

* *Cf.* Dendy's account of the larva of *Grantia labyrinthica* for similar cells, "On the Pseudogastrula stage in the Development of Calcareous Sponges," 'Roy. Soc. Victoria Proc.,' 1889, pp. 93—101.

of the larva, and its nucleus is usually, but not always, elongated in the same direction, so as to have a rod-like form. The whole structure, with pigment, lens-like body, and central granular cells, gives strongly the impression of a primitive, light-perceiving organ. The pigment itself is lodged in the inner ends of the ciliated and intermediate cells, and is, no doubt, the same pigment as that observed by Metschnikoff* and Schulze† in the inner ends of the ciliated cells in the larva of *Sycandra raphanus*. As the intermediate cells pass into the condition of granular cells, they leave the pigment behind, so that the pigment is thickest in the region of the intermediate cells, at the sides of the lens-like body.

The larva is thus composed of four kinds of cells, which may be termed the ciliated, intermediate, granular, and central cells. Since the intermediate cells are merely a transitional form between the ciliated cells proper and the granular cells, we have to reckon with three classes of cells only in the fully developed larva.

The fixation takes place by the anterior pole of the larva, and the granular cells grow round the ciliated cells. The metamorphosis is complete in a few hours. Sections of fixed stages of the first day of fixation (fig. 4) show them to be composed of two very distinct cell

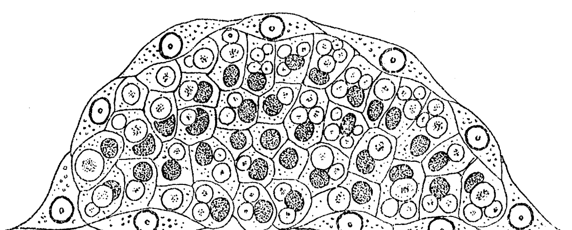

FIG. 4.—Section of larva shortly after fixation, the metamorphosis not quite complete.

layers: (1) a compact central mass of cells, easily recognisable, by their opaque, irregularly shaped nuclei and vacuolated cell protoplasm, as the former ciliated cells, surrounded by (2) *a single layer* of flattened epithelial cells, the former granular cells of the larva. No trace is to be found of the central cells, which appear to be thrown out, together with the pigment, at the metamorphosis. The inner mass is the future *gastral* layer of the sponge, the outer epithelium the future *dermal* layer.

* "Zur Entwicklungsgeschichte der Kalkschwämme," 'Zeitschr. f. Wiss. Zool.,' vol. 24, pp. 1—14, Taf. I.

† "Ueber den Bau und Entwicklung von *Sycandra raphanus*," *ib.*, vol. 25, suppl., pp. 247—280, Taf. XVIII—XXI.

The two component layers very soon begin to undergo changes of form and structure, which are best described separately, since the two layers develop more or less independently of one another, and a given stage in the development of one layer is not always found combined with one and the same stage in the development of the other.

The dermal layer becomes divided (fig. 5) into two kinds of cells: (*a*) cells which retain the original form and characters and remain on the surface, and (*b*) cells with smaller nuclei, which sink below the outer epithelium and form a scattered layer between it and the

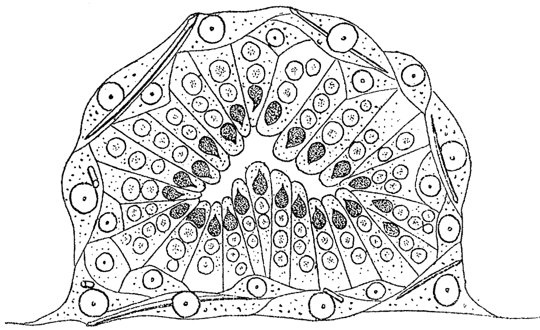

FIG. 5.—Section of stage about twenty-four hours after fixation. The left side is represented as slightly in advance of the right side.

gastral cells. The former (*a*) secrete each a single monaxon spicule, which appears first on the inner side of the nucleus, but soon grows out and projects free from the surface. The latter (*b*) unite into groups and secrete the triradiate spicules. The monaxons appear first, as in *Sycandra raphanus*,* and begin to appear about twenty-four hours after fixation, the triradiates about twelve hours later. The dermal layer has thus become divided into two parts, which gradually assume the adult characters. I have not observed the origin of the pores.

The gastral layer, at first a compact mass with no definite arrangement, soon begins to form a cavity (fig. 5). The cells assume a radiate arrangement, and a split-like lumen appears in the centre. Sometimes two or more such lacunar spaces arise, at first quite independent of one another, but later fusing to form a single gastral cavity, which soon becomes very large, causing the larva to increase considerably in size as a whole. At first the cavity is surrounded on all sides by gastral cells, but as it increases in size a spot appears where gastral cells are wanting, and the cavity is limited only by dermal cells (fig. 6). This is the region of the future osculum, and the dermal cells at this spot form the future oscular rim, where collar

* Metschnikoff, *loc. cit.*

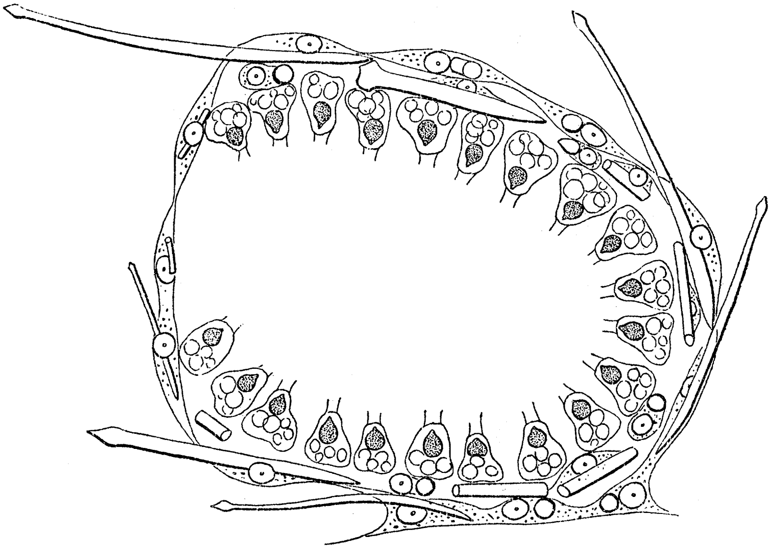

FIG. 6.—Section of stage of about the fourth day of fixation.

cells are lacking. The gastral cells are at first elongated, but later become shorter, and take on the characteristic appearance of collar cells. I have not been able to make out whether all the gastral cells become collar cells, or whether some of them do not become the wandering cells of the adult, which seems very probable. The osculum appears about the sixth day of fixation.

The Development of Leucosolenia cerebrum, *H*, L. reticulum, *O. S.*, *and* L. coriacea, Mont.

These three species have larvæ of the type with which we are familiar from the descriptions of Metschnikoff * and Schmidt,† namely, oval ciliated blastulæ, in which an inner mass is formed by immigration of cells into the interior. The process is most easily followed in the more transparent larva of *L. reticulum* (fig. 7), where the modification of ciliated cells into granular cells, and their subsequent immigration, takes place at the posterior pole. When the larva is ready for fixation, a considerable quantity of granular cells has been formed, though the cavity is far from being obliterated. In the opaque larvæ of *L. cerebrum* and *coriacea* the process is more

* "Spongiologische Studien," 'Zeitschr. f. Wiss. Zool.,' vol. 32, p. 362, Taf. XXIII.

† "Das Larvenstadium von *Ascetta clathrus* und *Ascetta primordialis*," 'Arch. f. Mikr. Anat.,' vol. 14, pp. 249—263, Taf. XV, XVI.

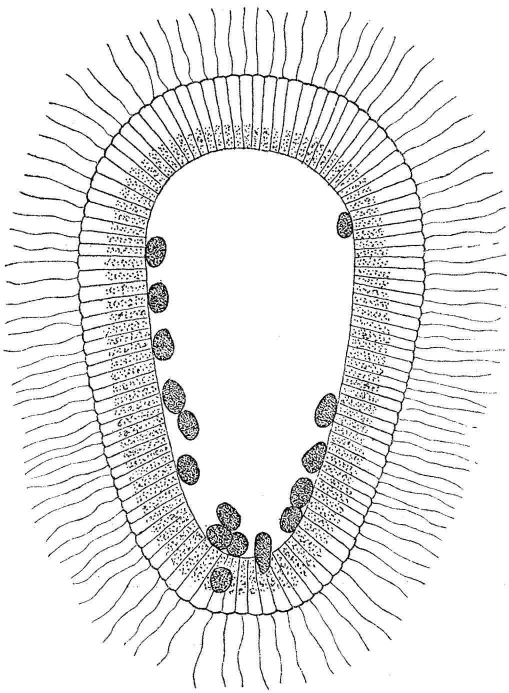

Fig. 7.—Optical section of larva of *L. reticulum*, first day, × 500.

difficult to follow, but in both immigration appears to take place from any point on the surface.

In *L. cerebrum* and *L. reticulum* the larva swims for about twenty-four hours at the surface, and as long at the bottom, and fixes on the third day. *L. coriacea*, on the other hand, is remarkable for its abbreviated larval period as compared with the two Mediterranean species, since the larva fixes in a few hours, a fact doubtless in connexion with its life between tide marks, where the violent currents to which it is exposed renders a very sheltered, and therefore limited, habitat necessary for so delicate an organism.

After fixation, the larva undergoes changes whereby the ciliated cells become surrounded by the formerly internal granular cells, so that the ciliated external layer of the larva represents the gastral layer of the adult, while the inner mass becomes the dermal layer; the reverse of what was supposed by Metschnikoff and Schmidt (*loc. cit.*) to take place.

In *L. cerebrum* I was able to observe the first appearance of the spicules. As in *variabilis*, the complete metamorphosis results in a stage in which the gastral cells form a compact internal mass, sur-

rounded by a single layer of dermal cells. Some of the cells of the dermal epithelium then form themselves into groups, usually of three cells, and each cell of such a group secretes the ray of a spicule. The first spicules are usually triradiate, but quite irregular in form, and at their first appearance they are quite superficial, their secreting cells forming part of the general epithelium, but later they become covered by the remaining epithelium, so that the dermal layer becomes divided into an internal connective tissue layer and an external flat epithelium. The process is essentially similar to that occurring in *variabilis*, except that in the latter the cells of the flat epithelium secrete each a monaxon spicule, which in *cerebrum* is not the case.

General Considerations.

The larva of *L. variabilis* is of interest as affording a transition from larvæ such as that of *L. reticulum*, to the amphiblastula larva of the Sycons. The larva of *reticulum* (fig. 7) is composed of (1) ciliated cells, comparable to those of the amphiblastula, of which some (2) at the hinder pole are undergoing modification, and may be compared with the intermediate cells, and of (3) internal granular cells comparable to the posterior granular cells of the amphiblastula. To obtain a larva like that of *variabilis* from the type represented by *reticulum*, we must suppose the large cavity of the latter reduced to the extent to which this has occurred in the former. Then the granular cells which are formed at the posterior pole must remain where they are, since the cavity is too small to contain them, and, as more ciliated cells are continually being modified around them, we get a larva with the three kinds of cells arranged as in *variabilis*. The central cells of *variabilis*—on the origin of which I have no observations to bring forward—are probably to be regarded as constituting a larval organ, a special adaptation of no importance for the postlarval development.

The development of both *reticulum* and *variabilis* points to an early stage in which the larva is composed entirely of similar and equivalent ciliated cells. I have not seen such a stage in any species, and doubt if it actually occurs in nature; it is more probable that the process of cell differentiation begins before the larva is hatched in all cases. In the absence of segmentation stages, it is impossible to decide this question; nevertheless, the facts seem to me to indicate, as the primitive larva in ascon phylogeny, a blastula composed of indifferent ciliated cells, in which a second type of cells (the future dermal layer) is formed by modification of certain of the cells. The collar-cell layer of the adult is derived directly from the primitive ciliated cells of the blastula.

Comparing, now, the larva of *variabilis* with that of *Sycon raphanus*,

as described by Schulze, it is obvious that the development is essentially similar in both, the chief difference being with regard to the periods at which the various events take place. In both the granular cells increase greatly in number, but in *raphanus* this takes place while the larva is still in the maternal tissues, as is obvious from Schulze's figures,* and the larva is hatched in a condition similar to that of *variabilis* when about to fix. In *variabilis* the granular cells do not surround the ciliated cells until after fixation; in *raphanus* this process is begun while the larva is still swimming, and the granular cells may even give rise to spicules (monaxons) during the free swimming period (Metschnikoff, *loc. cit.*). It is obvious that in *Sycon* we have before us a hastening and shortening of the development, and, allowing for these embryological adaptations, we are able to understand how, from a larva such as that of *reticulum*, there has arisen a type of development apparently so different as that of the *Sycon* amphiblastula.

The most important event in the post-larval development is the differentiation of the dermal layer into the outer epithelium and the inner connective tissue layer. This might seem at first sight to be a process comparable to the formation of a new layer, a mesoderm; so that from this period onwards the sponge would be a three-layered organism. I do not, however, take this view, for the following reason. The immigration of cells from the epithelium to form the layer of triradiates is not an event, like the formation of a germ layer, which takes places once and for all in the life cycle of an individual, but it goes on whenever new triradiates are formed. In adult ascons I have found that the triradiates and the basal rays of the quadriradiates arise from cells of the outer epithelium which migrate inwards and arrange themselves into groups to form spicules, each ray being secreted by one cell or by cells derived from the division of a single cell. In the adult also the nuclei of the spicule secreting cells diminish in size after quitting the epithelium. Hence in the development of the sponge also, I regard this process as one not of blastogenetic, but of histogenetic significance. The fact that in *variabilis* the epithelial cells also secrete spicules is to my mind a decisive proof of the unity of the dermal layer.†

* 'Zeitschr. f. Wiss. Zool.,' vol. 25, suppl., Taf. XX and fig. 3, Taf. XIX. Schulze refers this increase in the number of the granular cells to their multiplication by cell-division, but as the granular cells do not at the same time decrease in size, it seems more probable that their increase is due, as in *variabilis*, to their numbers being recruited from the clear (ciliated) cells.

† Schulze has also figured very clearly the relation of the dermal cells to the monaxon spicules, one spicule to each cell, in the young fixed stages of *Sycon raphanus* ('Zeitschr. f. Wiss. Zool.,' vol. 31, pl. XIX, figs. 10, 11), although he states in the text that the spicules arise in the hyaline substance between the two layers.

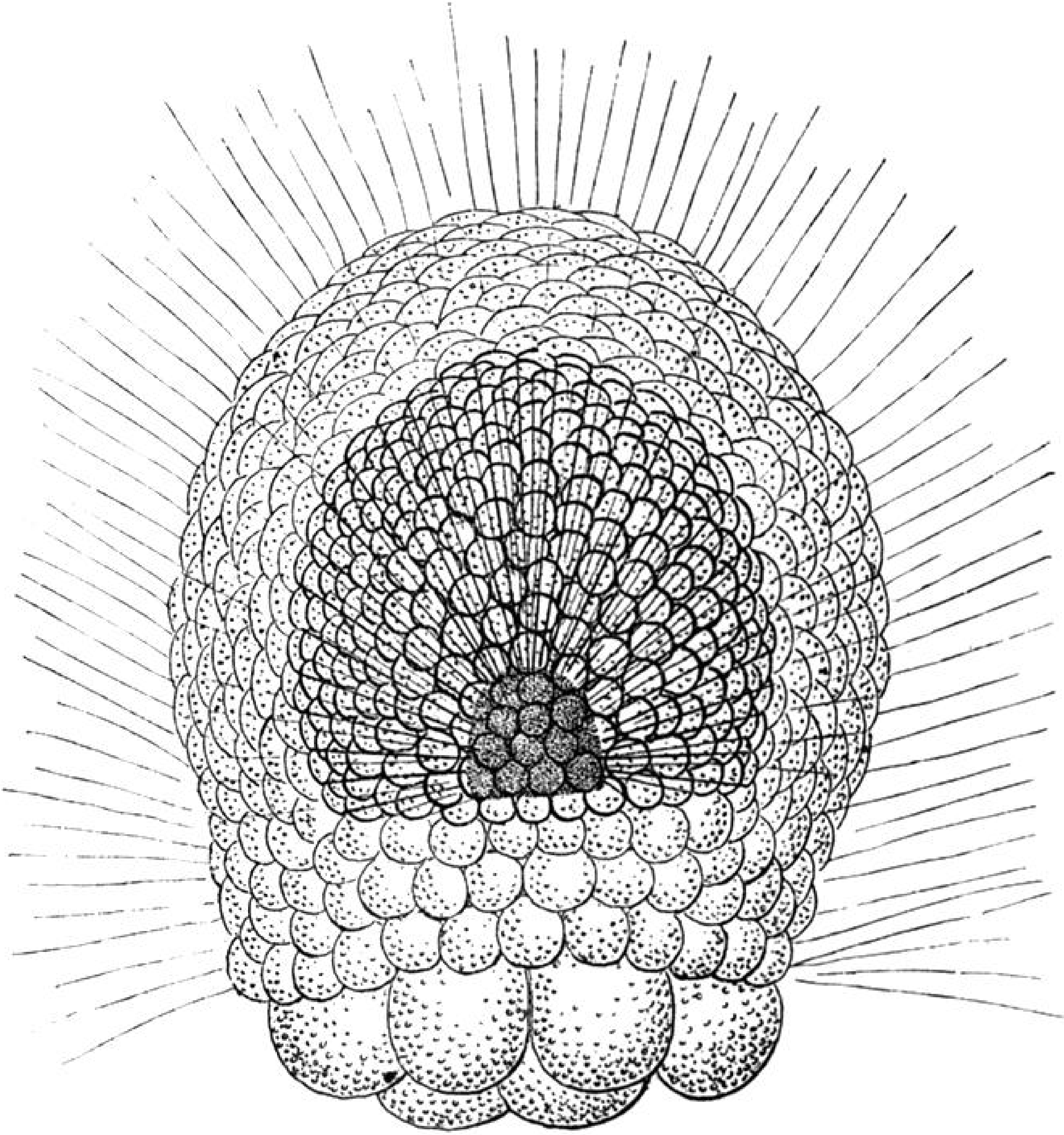

FIG. 1.—Newly hatched larva.

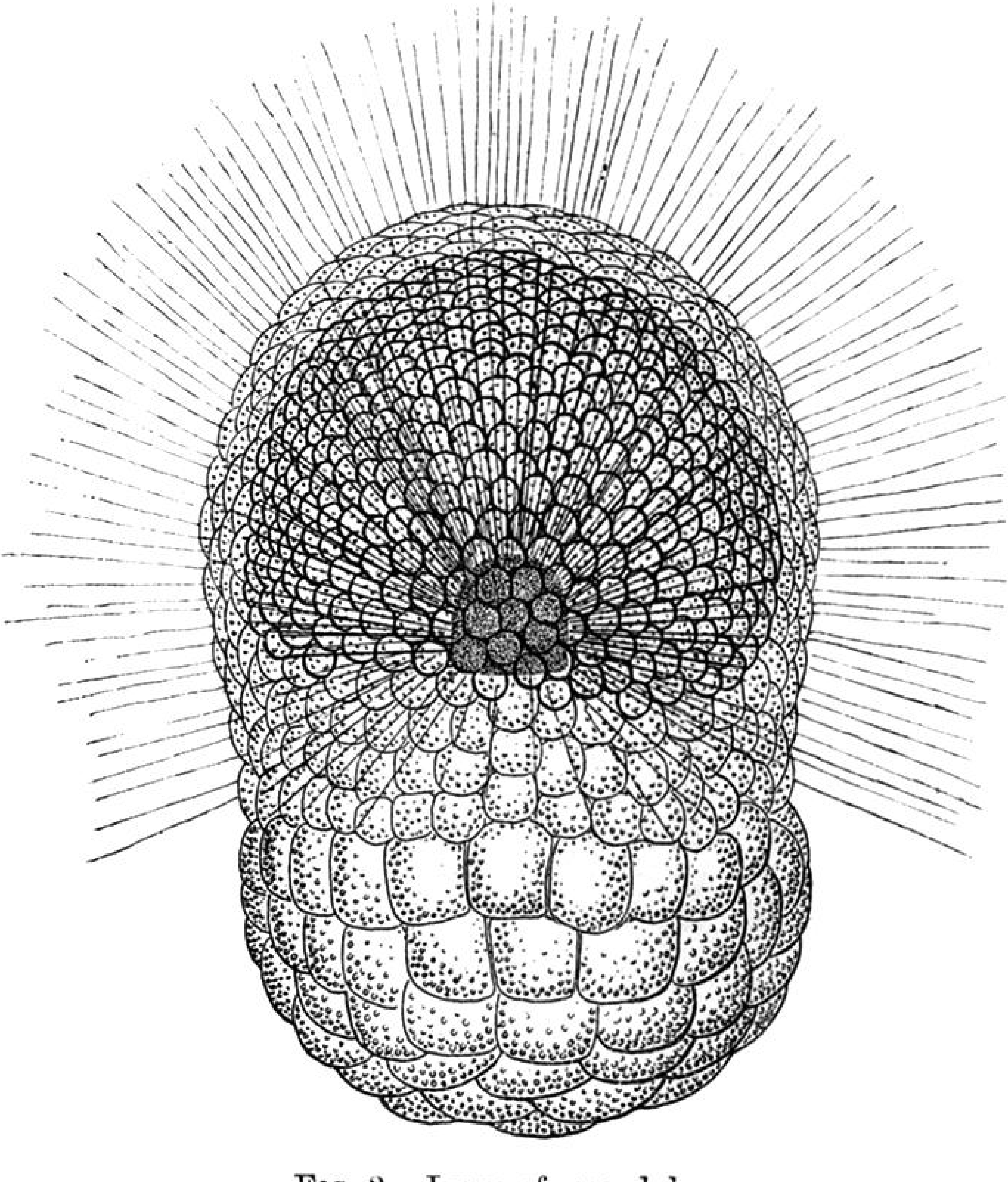

FIG. 2.—Larva of second day.

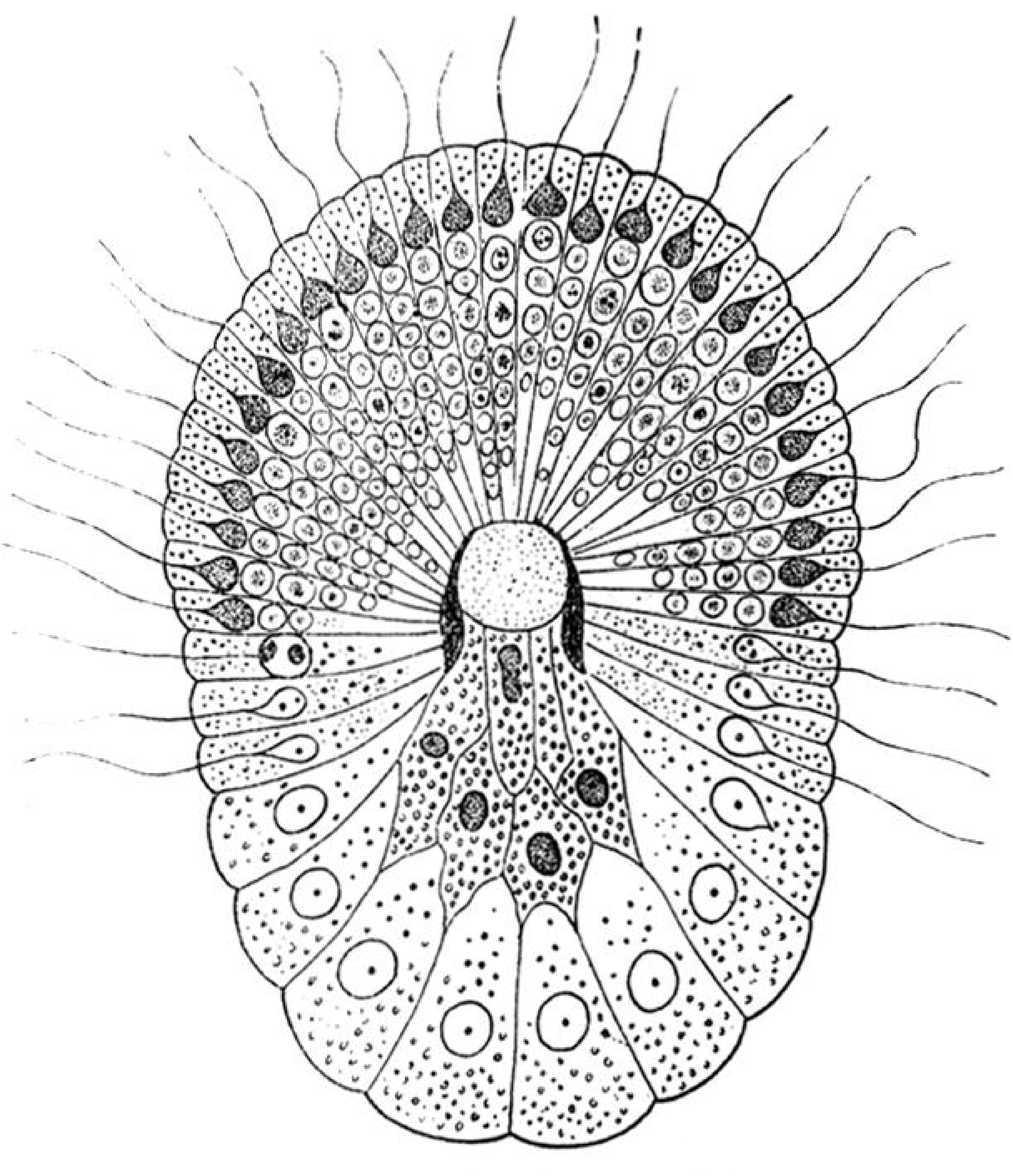

FIG. 3.—Longitudinal section of larva.

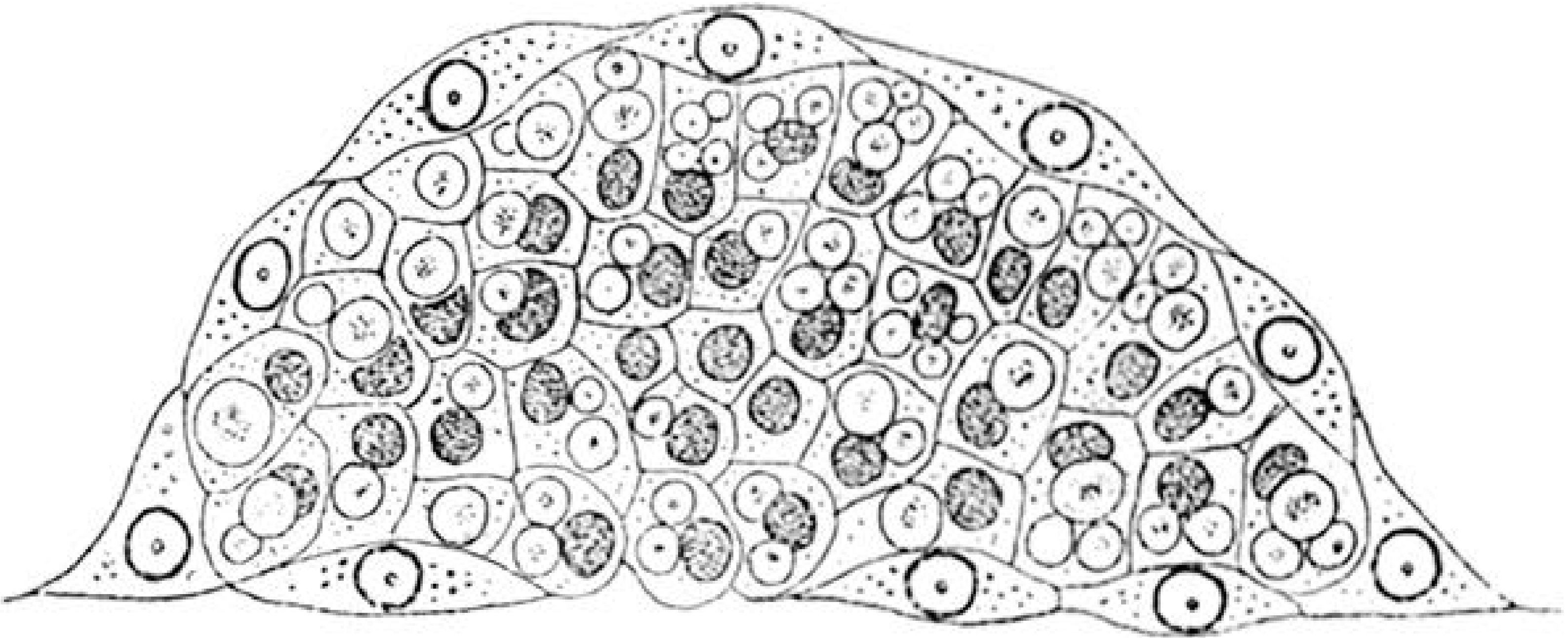

FIG. 4.—Section of larva shortly after fixation, the metamorphosis not quite complete.

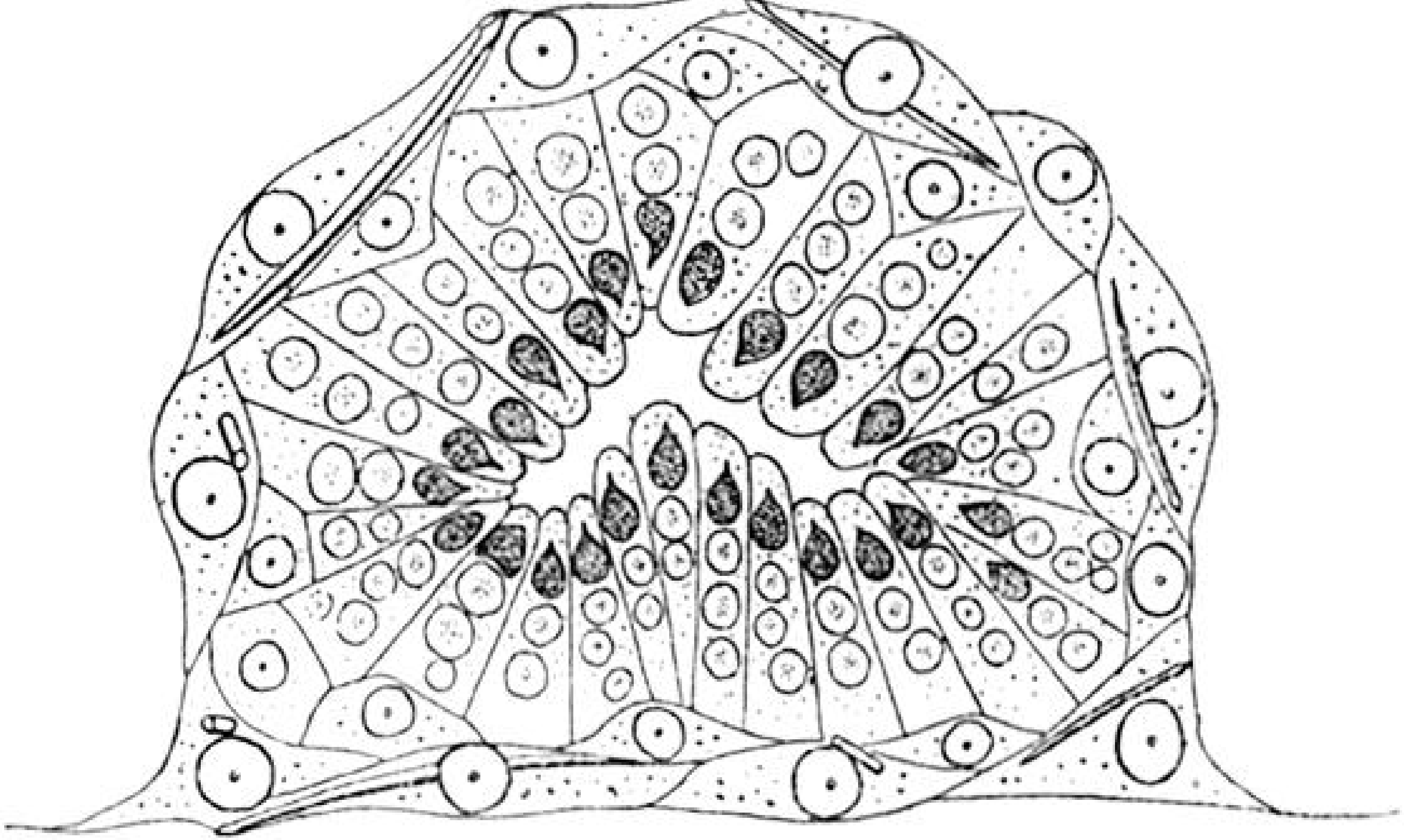

Fig. 5.—Section of stage about twenty-four hours after fixation. The left side is represented as slightly in advance of the right side.

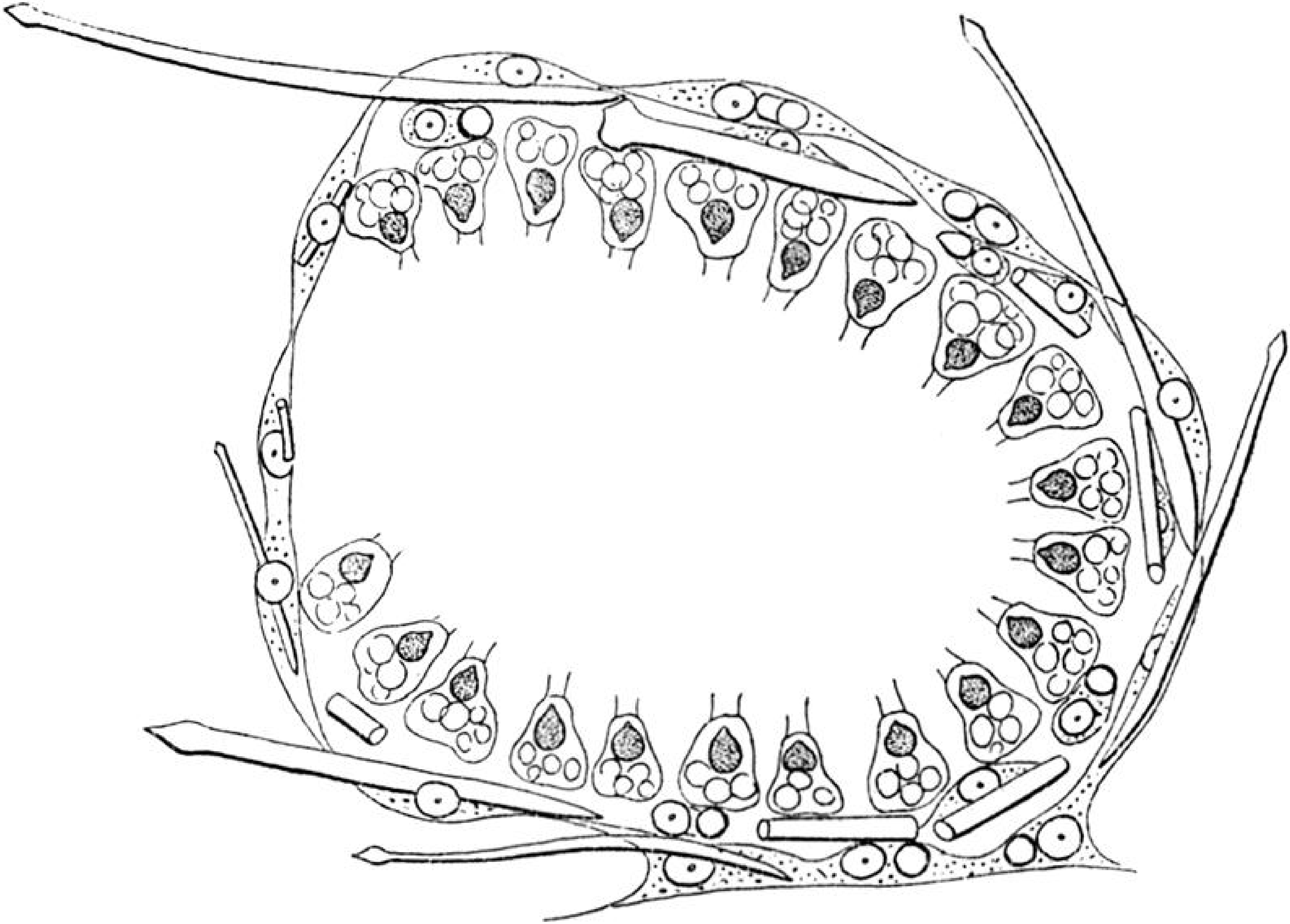

FIG. 6.—Section of stage of about the fourth day of fixation.

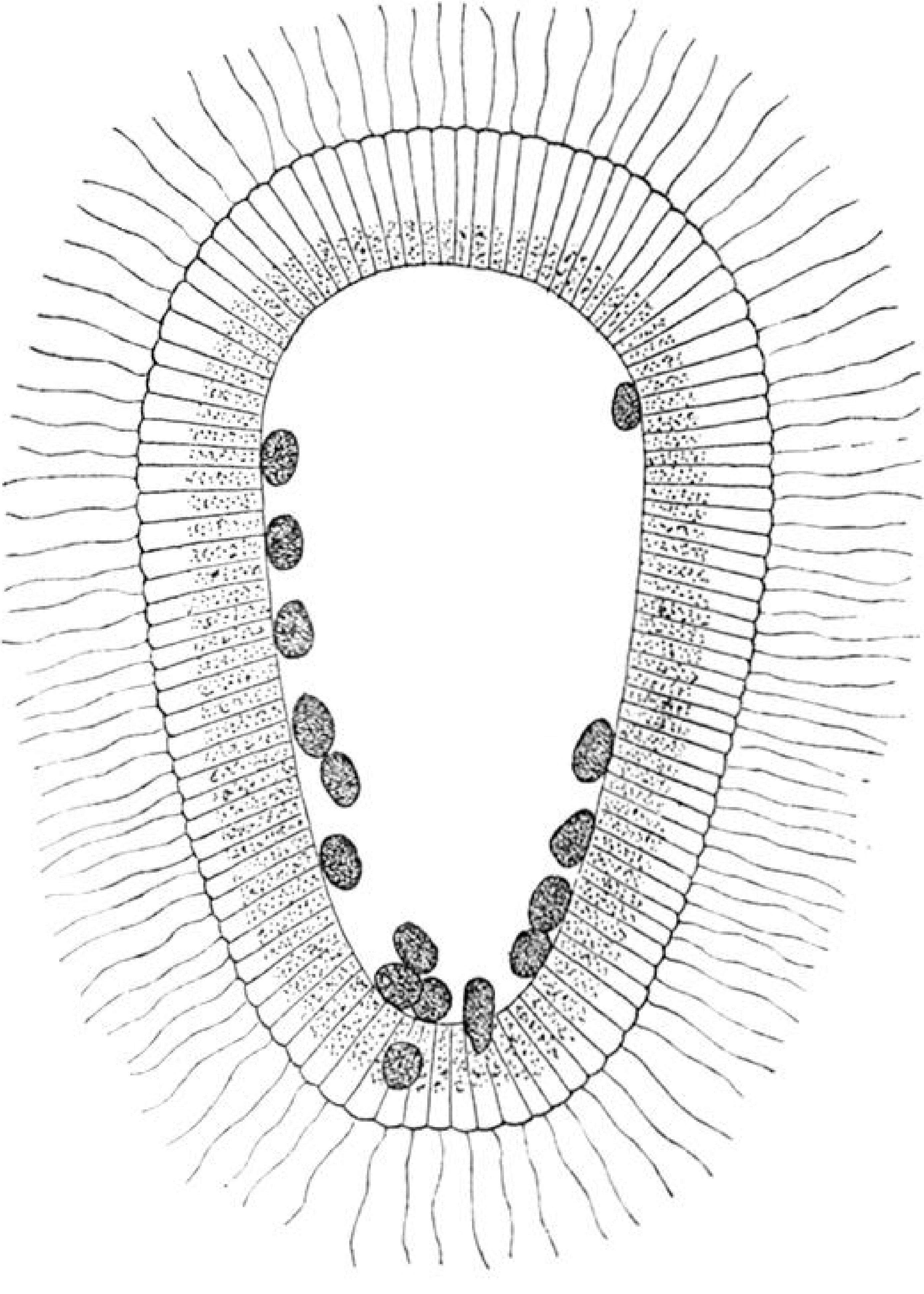

FIG. 7.—Optical section of larva of *L. reticulum*, first day, × 500.